L'ORÉAL®

March 03, 2000 2909 '00 MAR -7 A9:22 *via* **Fed Ex courier**

Docket Management Branch (HFA-305)
Food and Drug Administration, Room 1061
5630 Fishers Lane
Rockville, Maryland 20857

Re: Docket No. 78N-00388: Sunscreen Drug Products for Over-the-Counter Human Use

Dear Sir/Madam:

An accurate and reproducible method for determining a sunscreen product's UVA protection efficacy has been the subject of many proposals and comments to the Agency. Specifically, reference is made to the April 9, 1996 submission by the Cosmetic, Toiletry and Fragrance Association of a report entitled "Critical Wavelength Determination of the Evaluation of the UVA Efficacy of Sunscreen Products" and to the May 15, 1998 submission by L'Oréal Research to this docket. Therein we presented results of a series of investigations that revealed inadequacies associated with the Critical Wavelength method and a corresponding labeling scheme based on a "*broad spectrum*" designation. We showed that it does not provide a quantitative measure of the UVA protection afforded by a product as it merely measures the 'broadness' of the protection range. This is best illustrated in Figure 1 below.

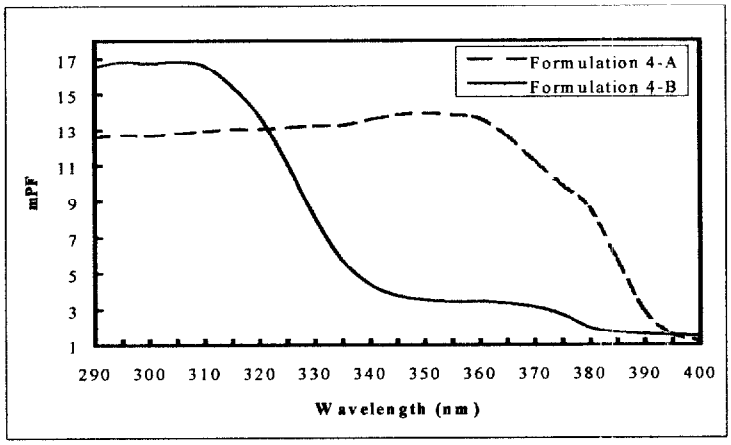

Figure 1: Monochromatic Protection Factor Curves of Two Prototype Formulations*.

Product Code	SPF (*in vivo*)	λ_c (nm)	UVA_cPF
4-A (408-312)	7.4	379	10.2
4-B (408-320)	7.5	372	5.2

* Reference is made to our May 15, 1998 submission to the Sunscreen Docket for specific data presented in this figure and corresponding summary data table.

78N-0038

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As we previously reported, it is evident from the shape of these two product absorbance curves that the formulations do not display the same protection level, with formulation 4-A being much more protective in the UVA range. However their respective critical wavelength value, each ≥ 370 nm would qualify both products for a "*broad spectrum*" labeling designation. As a result, this could be very misleading to consumers, particularly high-risk individuals who are very sun sensitive. These individuals as well as the average consumer would not have sufficient information to make the proper point-of-purchase selection of a sunscreen product.

While an *in vitro* method for the evaluation of product performance is highly desirable to industry, any such method should provide a realistic estimate of product protection against UVA effects on the skin and also take into account the photostability profile of the product. At the present time, no such *in vitro* method exists which has been shown to be validated against an acceptable *in vivo* method and we are left with *in vivo* methodologies* to assess this parameter of sunscreen protection.

In the context of global harmonization of clinical testing methods, L'Oréal Research / Cosmair Cosmetics Corp. is a proponent of the Persistent Pigment Darkening (PPD) method, implemented by The Japan Cosmetic Industry Association (JCIA) as the official method for assessment of the UVA efficacy of sunscreen products since 1996.

We would like to highlight to the Agency that the PPD method is very similar to the J&J UVA Erythema/Pigmentation (PFA) method. The biological end-point for the PFA method is the change in skin color, either erythema (redness) or tanning (browning) or a mixture of the two, observed 24 hours after exposure. The biological end-point for the PPD method is also a change in skin color observed at 2 or 24 hours after exposure. Both methods utilize light sources having identical emission spectra in the 320 nm to 400 nm range and differ only in the skin type of subjects used in the evaluation and the exact time for reading the corresponding responses.

As part of its continuing research initiatives in the area of sun protection, L'Oréal Research / Cosmair Cosmetics Corp. conducted a series of investigations presented herein to validate the PPD method for assessment of UVA product efficacy and also to assess its practicality in commercial testing laboratories.

The results of our investigations show that the PPD response is sensitive and specific to all UVA wavelengths and equally sensitive to the variety of UVA filtration schemes tested. Additionally, the PPD method is robust and reproducible and as a result of its similarity to SPF, the commercial testing laboratories may easily implement this test procedure. Based on our findings, we respectfully ask the Agency to consider the PPD method as another suitable *in vivo* method for determining UVA protection.

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* Reference is made to the Agency's position relating to UVA test methodology as described in the September 16, 1996 Federal Register (61 FR 48645 at 48651 to 48652), pertaining to avobenzone and also in a response to comments provided by Johnson & Johnson Consumer Products, Inc. (J&J) (Docket No. 78N-0038 Comments No. RPT5 and CR7). In these documents, it was stated that testing procedures similar to the methods described by J&J, R. W. Gange et al. (JAAD 15:494-499, 1986) and N.J. Lowe et al. (JAAD 15:224-230, 1987) are considered as appropriate for determining the UVA radiation protection potential of a sunscreen product.

L'Oréal Research is continuing its research in the area of UVA test methodologies and is presently developing an *in vitro* method that will be validated against the *in vivo* PPD method by using the PPD action spectrum. The same validation should be possible using the erythema action spectrum, for the *in vivo* J&J PFA method. In the context of new sunscreen product development, having both validated *in vivo* and *in vitro* UVA methods allows the greatest flexibility to choose the most appropriate method depending on the test objective, e.g., water resistant UVA-PF determinations, formulation screening, etc.

Commensurate with advances in sunscreen formulation technology, we believe that the method chosen for evaluation of UVA product performance should accurately and quantitatively measure the corresponding protection benefits. Furthermore any labeling scheme developed should also differentiate UVA product benefits to the consumer so as to ensure choice of a correct protection level, i.e. both UVA and UVB, for an individual's health needs and/or sun exposure habits. We will continue to work with the Agency and our industry forum to advance this area of mutual interest.

Should you need additional information or have any questions on the data in this submission, please contact me at 732-499-6600.

Sincerely,

Cheryl M Sanzare

Cheryl M. Sanzare
Assistant Vice President, Drug Regulatory Affairs
L'Oréal Research / Cosmair Cosmetics Corp.

FDA Desk Copies
C. Ganley, M.D. (HFD-560)
D. Dobbs (HFD-560)
J. Lipnicki (HFD-560)
J. Wilkin, M.D. (HFD-540)

Cc: A. J. Penicnak, Senior Vice President, Corporate Scientific, Cosmair Cosmetics Corp.
C. Corbett, Associate General Counsel, Cosmair, Inc.

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2904 '00 MAR -7 A9:21

March 6, 2000

Ms. Williams
F.D.A. Room 1061
Docket Management Branch (HFA-305)
5630 Fishers Lane
ROCKVILLE, MD 20852

Re: Corrected cover letter Docket No. 78N-00388: Sunscreen Drug Products for Over-the-Counter Human Use

Dear Ms. Williams:

As per our phone conversation of today, please replace the cover letter sent to you in triplicate on March 3rd, 2000 with the enclosed one, again in triplicate, as there was an inadvertent typo on page 3 of 3.

Sincerely,

F. Miele

Françoise Miele
Regulatory Assistant
L'Oréal Research - Cosmair Cosmetics Corp.

78N-0038

Sup 30

EVALUATION OF THE *IN VIVO*

PERSISTENT PIGMENT DARKENING METHOD

FOR DETERMINATION OF UVA PROTECTION EFFICACY

L'Oréal Research
Cosmair Cosmetics Corp.

EVALUATION OF THE *IN VIVO* PERSISTENT PIGMENT DARKENING METHOD FOR DETERMINATION OF UVA PROTECTION EFFICACY

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EVALUATION OF THE *IN VIVO* PERSISTENT PIGMENT DARKENING METHOD FOR DETERMINATION OF UVA PROTECTION EFFICACY

INTRODUCTION

The advent of new and highly effective UV filters, while initially developed as a means of preventing sunburn, represents a major advance in the ability of sunscreen products to protect against many adverse effects including erythema, premature skin aging, photosensitivity disorders and some forms of skin cancer While the risk for sunburn primarily from UVB (290-320nm) exposure has been widely recognized by the scientific community, recent studies[1-5] have shown that UVA (320-400nm) radiation contributes to the risk by inducing an immunosuppression reaction[6,9]. Increasing concern about these effects has led to the development of sunscreens that effectively attenuate and offer extended protection in the UVA range.

The accepted method for evaluating protection by sunscreens is the SPF method however this method does not assess the complete UVA protection of a product[29]. Therefore, various *in vitro* and *in vivo* methods have been proposed with enhanced selectivity in the UVA range for evaluating the UVA protection offered by sunscreen products.

In vitro test methods, based on spectroradiometric analysis of products applied to an inert substrate (e.g. Transpore® tape, quartz plate), are of interest to the cosmetic and OTC drug industry because they are convenient and cost effective for product development However, the major drawback of *in vitro* test methodologies is their inability to predict product performance on human skin.

In vitro methods proposed to date include the UVA Percentage Protection (APP)[10], the Broad Spectrum Classification (Diffey method)[11,12], the Boots "Star System" and the percent transmittance of a solution or a thin film (Australian/New Zealand standard)[13]. A modification of the second method, i.e. the Modified Diffey Consensus Method (also referred to as the Critical Wavelength method) was proposed to the FDA by the CTFA[14,15]. This last method relies on a mathematical calculation using UV transmission data derived from spectroradiometric measurements However, none of these methods utilize a biological action spectrum nor a light source emission spectrum.

Data submitted on May 15, 1998, to the FDA by L'Oréal Research/Cosmair Cosmetics Corporation show that the Critical Wavelength Method, based on an arbitrary non-biological cutoff criteria, fails to provide an adequate measure of a product's UVA erythemal protection efficacy when compared to the product's UVA erythemal index[16]. This flaw results in a ranking of sun products that is distorted when compared to their actual efficiency in the UVA waveband The system is further biased because it implies

an increase in the UVB protection with a non proportional increase in UVA protection.

Therefore, despite their ease of use and apparent reproducibility, existing *in vitro* methods lack in their ability to predict the biological response of the protected skin to UV exposure.

In vivo methods for the determination of UVA protection have been described in the scientific literature. These include: the Phototoxic Protection Factor method (PPF)[17,18], the Immediate Pigment Darkening (IPD)[21,22] method, the UVA Protection Factor (PFA)[19-20] also known as UVA Erythema-Pigmentation method, and the Persistent Pigment Darkening (PPD)[23,24] method (refer to Appendix I).

These four *in vivo* methods have different end-points for assessing UVA protection efficacy: the phototoxic method is only relevant for sensitized people and the IPD method overestimates the UVA protection due to the non-compliance with the reciprocity law[23] .♠ The other two methods have similarities: the action spectra of erythema and persistent pigmentation, in the range of 330-400 nm, are parallel[23]. Since the action spectra of both responses are so similar[19,20,23] in the UVA range they are expected to yield similar estimates of protection.

In the May 12, 1993 OTC Sunscreen Drug Products Tentative Final Monograph and its subsequent amendment (October 22, 1998) and enforcement policy (April 30, 1997) concerning UVA protection, FDA has recognized two *in vivo* methods, i.e., the PPF method and the PFA method. The latter method is very similar to the PPD method as its biological end-point is the change in skin color, either erythema (redness) or tanning (browning) or a mixture of the two, observed within a preset time interval after exposure (two or twenty four hours). The UVA light sources utilized in the PPD and PFA methodology have identical emission spectra in the 320-400nm range.

As the action spectra of UVA damage have not been established, selection of an *in vivo* method should be based on a measurable skin response, with a corresponding action spectrum covering the entire UVA range. Furthermore, the skin response evaluated should comply with the reciprocity law[23].♠ Additionally, the method should utilize a

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♠ The reciprocity law applies to photochemistry and photobiology when a given effect in a substrate is induced by a given dose of light radiation according to the equation D = [F] [t]; where a light dose [D (J/m^2)] is obtained from a light source of a known intensity [or fluence rate – F(W/m^2)] for a given duration of exposure [t (s)]. For example, this law is applicable to photographic film exposure. With a small aperature (low fluence rate), longer exposure times (t) are necessary for the film to capture the image. Reciprocally, with a large aperature (high fluence rate), exposure times (t) are very short. The reciprocity law holds true in that no matter which light/exposure combination used (small/long or large/short), the result is the same, a photograph of the image.
This rule should also hold true for the skin responses used in sunscreen SPF (erythema) and UVA-PF (PPD) testing in order to avoid significant bias (over- or under-estimation) of the PF values and to limit undesirable variability. The two main reasons for variability are : the intensity of the UV light reaching the skin is lower on protected skin (longer exposure time until MED) than on unprotected skin (shorter exposure time until MED) and the variability in the output intensity of the UV solar simulators used in test laboratories.

standard light source having a uniform output throughout the UVA range of wavelengths and one which does not include UVB or visible radiation.

The Japan Cosmetic Industry Association (JCIA) has selected and implemented the PPD method (refer to Appendix I) as the official method for assessment of the UVA efficacy of sunscreen products since January 1996[24]. The same method has also been in use by LOréal to evaluate and label the UVA protection of sunscreens since 1993 for products sold in the EU market.

The series of investigations presented herein, each utilizing the PPD method for assessment of UVA product efficacy, will confirm the validity of this method as well as its suitability for use in the commercial testing environment.

OVERALL OBJECTIVES

A series of investigations were undertaken to validate the PPD method for accurately predicting the UVA efficacy of a sunscreen product. The investigations may be classified in two groups:

Part 1: Performance of the proposed PPD method
- Part 1A: Calibration of the PPD method as an *in-vivo* UVA dosimeter;
- Part 1B: Sensitivity and Specificity of the PPD method;

Part 2: Evaluation of the reproducibility of the PPD method at three commercial testing laboratories.

THE LIGHT SOURCE

The design of the light source is of principal importance when using the PPD method. The light source must have a uniform output throughout the UVA range (320-400 nm) with additional filters to exclude the UVB and visible wavelengths from the output spectrum. The UVB and visible wavelengths contribute erythema and a pigment response, respectively, which could confound the results. The source chosen (described in detail in the Materials and Methods sections in Parts 1 and 2) was a Xenon arc solar simulator filtered at both the short and the long wavelengths to produce a uniform output throughout the UVA range with no contributions from the UVB or the visible spectra.

PART 1 : PERFORMANCE OF THE PROPOSED PPD METHOD

PART 1A: CALIBRATION OF THE PPD METHOD AS AN *IN VIVO* UVA DOSIMETER.

Calibration of the PPD Method using Neutral-Density Physical Filters

Following the procedure recommended by the Colipa SPF test method (refer to Appendix II), the PPD method was calibrated using neutral-density physical filters as physical standards of UV filtering efficacy. The aim was to establish that PPD is an accurate and sensitive dosimeter for measuring the UVA radiation that enters the epidermis.

The four neutral-density physical filters (MTO Neutravex®) which served to calibrate the method are made of a uniform metallic layer deposited between two silica plates mounted in a frame. These optical filters present a neutral absorbance throughout the solar UV and visible range and their reference PF values in the UVA waveband, extending from 3 to 20, were determined spectroradiometrically using a derived Diffey technique[11,13] (Optometrics SPF-290 analyser, Bentham DL150 spectroradiometer and transmission device) and radiometrically by global UVA transmission according to the technique described in the Australian standard Appendix C[13] (Solar-Light-Co PMA2110F flat UVA sensor and Oriel 81292 6x6" xenon source filtered with Schott WG335 / 3 mm and UG11 / 1 mm filters). Since the absorbance through these filters is constant across the entire UV range, the *in-vitro* UVA-PF values obtained for these filters should be similar to their respective *in vivo* SPF or PPD UVA-PF values.

Each of the filters were affixed onto a template with adhesive tape which was then placed on the subject's back in order to avoid direct contact of the filter with the skin[23]. Particular attention was given to maintaining the light-guides of the UVA source at the same distance from the skin for exposures with and without the presence of the neutral density filters. The JCIA test procedure (refer to Appendix I) was followed and PPD UVA-PF values were determined from visual observations taken 3 ± 1 hour post exposure.

Neutral-density Filter Reference Number	*In vitro* UVA-PF (Radiometric Value)	*In vitro* UVA-PF (Spectroradiometric Value)	Mean *In vivo* PPD UVA-PF Value (SD, n)
#7200	3.0	3.2	**2.4** (0.3, 10)
#7817	6.0	7.0	**5.6** (1.2, 7)
#7784	8.4	10.4	**7.4** (1.2, 10)
#12490	15.3	19.5	**13.9** (2.9, 10)

Table 1: PPD UVA-PF Values with Neutral-Density Physical Filters

The results presented in Table 1 and Figure 1 demonstrate that the PPD response of the skin is an accurate and linear dosimeter for UVA radiation in the epidermis, thus confirming calibration of the PPD method. [Refer to Appendix IV for individual subject data from PPD evaluations.]

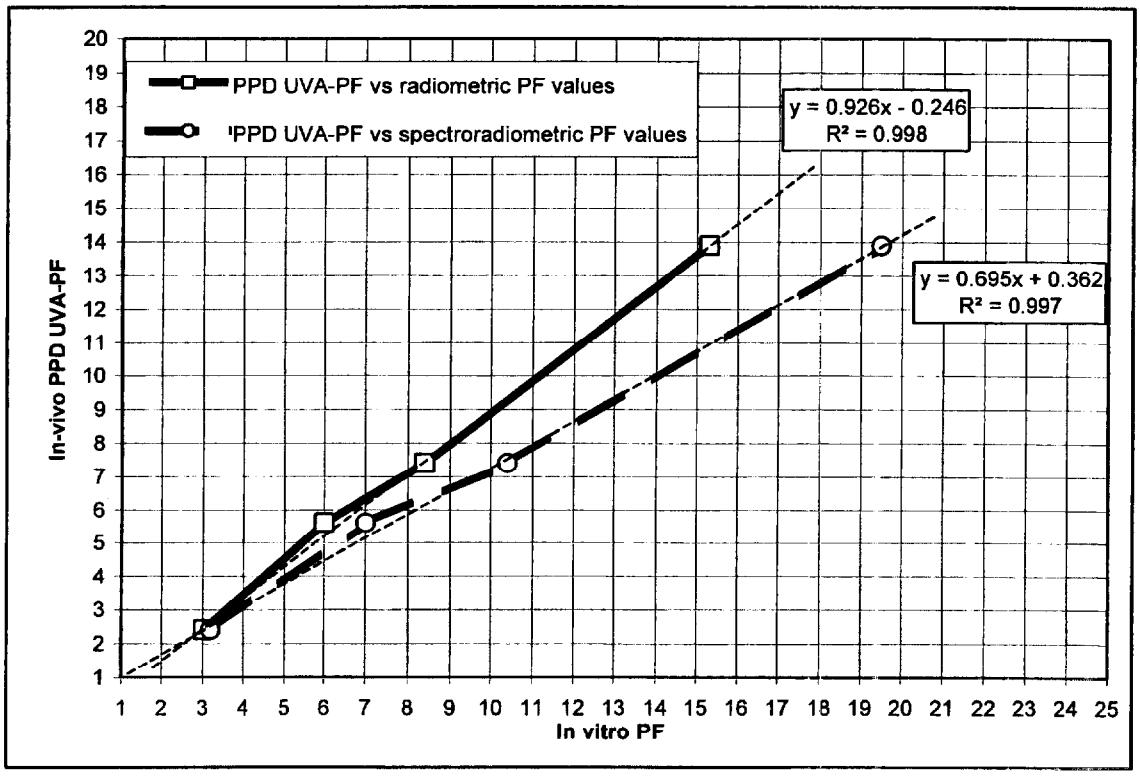

Figure 1: Calibration of the PPD Method using Neutral-Density Physical Filters

PART 1B: SENSITIVITY AND SPECIFICITY OF THE PPD METHOD

OBJECTIVE

In a series of investigations presented in this section, the sensitivity and specificity of the method to discriminate between sunscreen products (or laboratory formulations) providing different levels of UVA protection was evaluated The sensitivity of the PPD method was determined by assessing its ability to discriminate concentration-effects of UVA filters and combinations thereof. The specificity of the PPD method to evaluate differences in UVA protection levels provided by various UVA filters, as well as combinations of UVA filters, was tested independent of the contribution of UVB filters.

MATERIALS AND METHODS

Study Design

This series of investigations were conducted in the Applied Research and Development Laboratories of L'Oréal in Clichy, France . The JCIA test procedure was followed for each investigation. The sunscreen standard control defined in the JCIA method was formulated in the L'Oréal laboratories. It was tested to show whether the results obtained with the L'Oréal sunscreen standard control conform to the specifications of the JCIA standard.

Subjects

Members of a volunteer panel were selected to participate in these investigations if they met the selection criteria outlined in the JCIA test procedure. Subjects included men and women with Fitzpatrick Skin Types II, III and IV[25].

Ultraviolet Light Source and Calibration

The UVA light source utilized in these studies was filtered at both the short and long UVA wavelengths to provide a continuous emission spectrum in the UVA range between 320 and 400nm, representative of the UVA portion of the standard sunlight spectrum [26].

The light source consisted of a 150-W Xenon arc solar simulator (Multiport Model 600 or 601, Solar Light Co., Philadelphia, PA), equipped with a dichroic mirror, a Schott WG335 / 3 mm filter to eliminate UVB radiation and a Schott UG11 / 1 mm to eliminate visible and infrared radiation. The output spectrum of the light source was measured by a qualified expert (refer to Appendix III) as specified in the COLIPA SPF test method (refer to Appendix II). This spectrum is shown below in Figure 2.

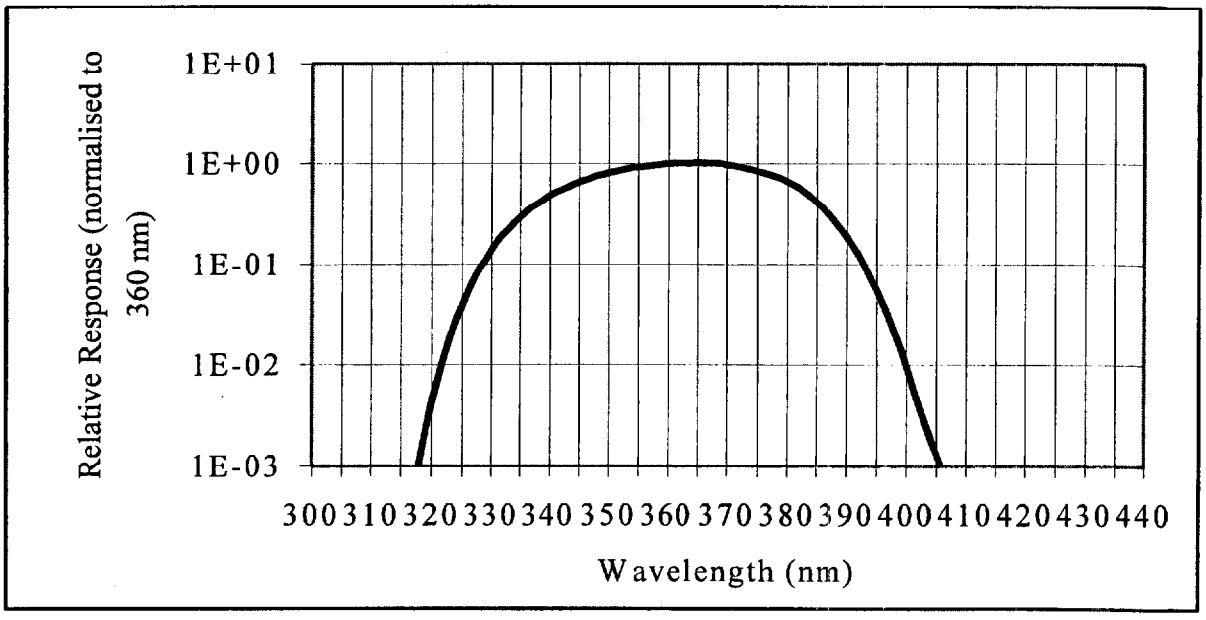

Figure 2: Emission Spectrum of the UVA Source. The source was a Xenon arc lamp filtered with WG335 / 3 mm and UG11 / 1 mm.

The UVB component of the light source did not exceed 0.1% of the UVA flux to minimize the erythemal response. The percentage ratio of UVAII (320-340 nm) to UVA irradiance was 10%, similar to standard sunlight (refer to Appendices I and II). The UVA irradiance at the surface of the skin was approximately 60 mW/cm².

Test Products

Various laboratory formulations and commercial products purchased in 1997/1998 were selected to assess the sensitivity and selectivity of the PPD method to reliably evaluate their UVA protection efficacy.[Φ] The laboratory formulations consisted of oil-in-water emulsions containing different concentrations and combinations of sunscreen ingredients as described in Tables 3 to 6.

Test Procedure

Test sites (40 cm²) were selected on the back of volunteers with skin phototypes II to IV[25], between the waistline and scapulae and lateral to the midline. Test products were applied in the amount of 2.0 mg/cm² and spread uniformly over the test site using a fingercot. The products were allowed to dry for 15 minutes prior to UVA exposure.

The UVA-PF of a given product was determined by exposing a set of six sub-sites (each 0.8 cm in diameter), on an unprotected and sunscreen protected area of the volunteer's back, to respective series of increasing UVA doses from the solar simulator. The exposure doses were calculated using a geometric series wherein each exposure was twenty five percent greater than the previous one. The series of six UVA doses covered an energy range of 8 to 25 J/cm² on unprotected skin for all volunteers, and these exposure doses were multiplied by the estimated UVA-PF of the product to calculate the exposure doses for the protected sites.

Φ **List of Commercial Products Tested (1997/1998)**

US PRODUCTS	LABELED SPF
Coppertone Waterproof – Moisturizing Suntan Lotion	4
Coppertone Sport – Ultra Sweatproof	8
Coppertone All Day – Moisturizing Sunblock Lotion	15
Coppertone All Day – Moisturizing Sunblock Lotion	30
Coppertone All Day – Moisturizing Sunblock Lotion	45
CTFA Sunscreen Standard	15
Zinc Oxide 20% USP	-
Avon Age Block	15
Le Mirador – Triple Action Revitalizing Moisturizer	15
Shade UVA Guard	15
EUROPEAN PRODUCTS	**LABELED SPF**
Ambre Solaire Lait	15
Ambre Solaire – Sunblock Milk	30
Nivea Sun – Moisturizing Sunblock Lotion	30
Oil of Olay Complete Care	15

The minimal pigmenting dose (MPD) was assessed when the PPD response is stabilized, i.e. 3 ± 1 hours post UVA exposure. This time lag prior to assessment of the PPD response began following irradiation of the last test sub-site. Visual evaluation was performed in sufficient and uniform illumination.

The minimal pigmentation dose for unprotected skin (MPDu) and for protected skin (MPDp) was visually determined simultaneously in a paired comparison. MPDu and MPDp are defined as the quantity of radiant UVA energy required to elicit the first perceptible, unambiguous pigmented reaction with clearly defined borders. The pigmentation threshold taken for the MPDu and MPDp determination should be identical for the protected and unprotected areas and should correspond to an average colorimetric luminance differential[23] of $\Delta L^* = -1.2$. The UVA dose required to induce a minimal persistent pigmentation is about 15 J/cm² and represents approximately the amount of UVA exposure received during one hour of noon sun exposure, i.e., 12 PM on a clear June day at 40 to 50° latitudes (e.g. Vancouver, Seattle, Toronto, Boston, Chicago).

Calculation of the UVA-PF

An individual's (*i*) PPD UVA-PF value, i.e., UVA-PF*i*, for a product is defined as the ratio of the minimal pigmenting dose on protected skin (MPDp) to the minimal pigmenting dose on unprotected skin (MPDu) of the same subject as follows:

$$\text{UVA-PF}i = \frac{\text{MPDp}}{\text{MPDu}}$$

The UVA-PF for the product is the arithmetic mean of the UVA-PF*i* values obtained from at least 10 subjects. The standard deviation was calculated as a measure of the variance of the measurements.

RESULTS

Tabulated individual PPD UVA-PF data for each product assessment are presented in Appendix IV, with their mean, standard deviation (SD) and standard error of the mean (SEM = SD / $\sqrt{n}$, n being the number of volunteers used).

Determination of the UVA-PF of the JCIA Sunscreen Standard Control using PPD

Table 2 summarizes the mean UVA-PF values obtained in our laboratory from the sunscreen standard control recommended by the JCIA test procedure (Appendix I) in a series of measurements.

Test	Mean UVA-PF	n	SD	SEM
Series 1	**4.8**	10	1.3	0.4
Series 2	**5.5**	10	1.4	0.4
Series 3	**4.7**	10	1.0	0.3
Series 4	**3.9**	11	0.9	0.3
Grand mean	**4.7**	(41)	1.1	0.4

Table 2: UVA-PF of the JCIA Sunscreen Standard Control

These values are within the range of values or slightly higher than the upper limit (4.76) of the range specified by the JCIA PPD UVA-PF test method (3.75, SD: ± 1.01). [Refer to Appendix IV for individual subject data.]

Determination of the Effect of Concentration of Organic UVA Filters on UVA-PF

Studies were conducted to assess the sensitivity and selectivity of the PPD UVA-PF method to distinguish among increasing concentrations of three filters effective in the UVA waveband (320-400nm). The filters chosen included oxybenzone (benzophenone-3, OXY), a UVB/UVA filter with contribution in the short UVA waveband and maximum absorption at 323nm, tested at concentrations of 0.0 (vehicle), 1.0, 3.0 and 5.0%; ecamsule (terephthalylidene dicamphor sulfonic acid, TDSA, Mexoryl®SX), a broad spectrum UVA filter with maximum absorption at 345nm, tested at concentrations of 0.0 (vehicle), 2.0, 4.0 and 8.0%; and avobenzone (butyl methoxy-dibenzoylmethane, BMDM, Parsol®1789) with maximum absorption at 357nm, tested at concentrations of 0.0 (vehicle), 1.0, 3.0 and 5.0%. The mean PPD UVA-PF values for the products at the concentrations listed above and their corresponding vehicle are presented in Table 3. The effect of concentration on the UVA-PF determined by the PPD method is shown in Figure 3. [Refer to Appendix IV for individual subject data.]

Product Code	UVA Filter Composition	Mean UVA-PF Value (SD, n)
A-L	Vehicle	**1.1** (0.2, 20*)
AD	OXY 1.0%	**1.8** (0.4, 10)
AE	OXY 3.0%	**3.1** (0.6, 10)
AF	OXY 5.0%	**4.0** (1.3, 10)
L-L	TDSA 2.0%	**3.6** (1.1, 10)
M-L	TDSA 4.0%	**5.0** (1.3, 10)
N-L	TDSA 8.0%	**10.9** (3.2, 10)
B-L	BMDM 1.0%	**2.2** (0.5, 10)
C-L	BMDM 3.0%	**4.0** (0.8, 10)
D-L	BMDM 5.0%	**4.6** (1.2, 10)

*The vehicle A-L was tested in two separate UVA-PF evaluations.

Table 3 - Dose-Effect of UVA Filters Alone

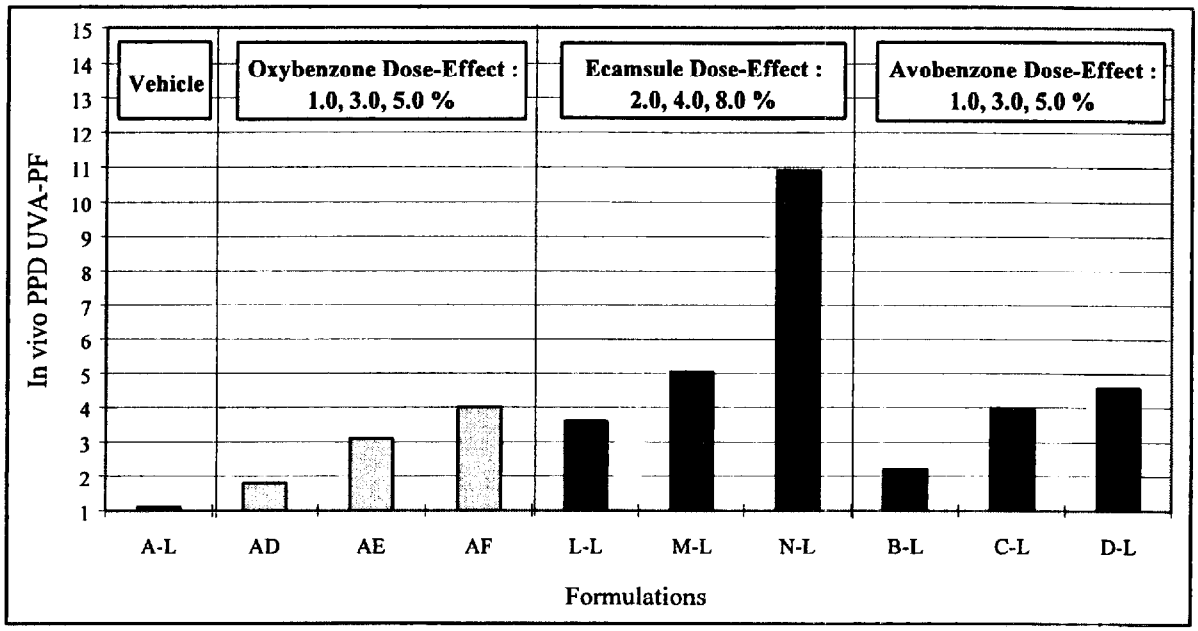

Figure 3 - Dose-effect of UVA Filters Alone (Oxybenzone, Avobenzone and Ecamsule)

These three filters alone, in their respective waveband, yielded a significant dose-dependent relation between UVA-PF protection and filter concentration

Determination of the Effect of Concentration of a Mineral Filter, Zinc Oxide, on UVA-PF

The PPD method was also used to evaluate the protection efficacy of a physical filter, zinc oxide (ZnO), in the UVA spectrum. Formulations containing ZnO at increasing concentrations were prepared. The results of these studies are presented in Table 4 and Figure 4. [Refer to Appendix IV for individual subject data.]

Product Code	UV Filter Composition	Mean UVA-PF Value (SD, n)
Y-L	ZnO 2.0%	**1.5** (0.4, 10)
Z-L	ZnO 4.0%	**1.8** (0.5, 10)
AZ-L	ZnO 8.0%	**2.5** (0.8, 10)
G	ZnO 20.0%	**3.1** (0.8, 10)

Table 4: Dose-Effect of Zinc Oxide

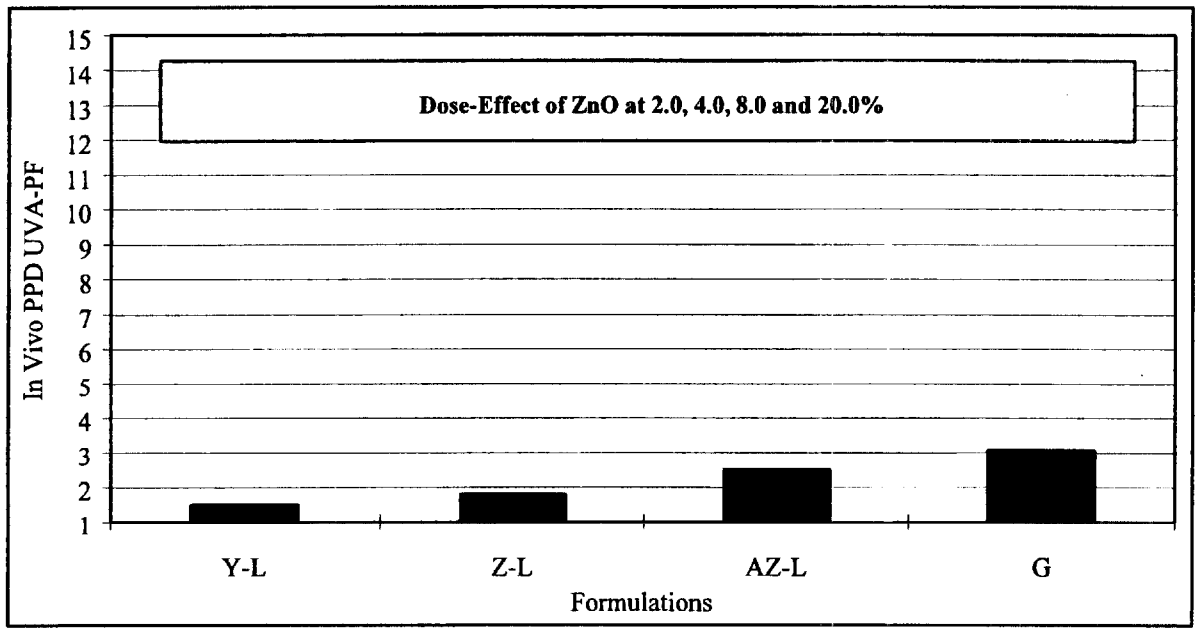

Figure 4 - Dose-effect of Zinc Oxide

The sensitivity of the PPD method in evaluating the UVA protection of zinc oxide alone (dose-effect) is demonstrated. The UVA-PF values increased from 1.5 to 3.1 with increasing concentration of zinc oxide in the formulations.

Determination of UVA-PF of UVA filters when used in combination with UVB filters

Sunscreen formulations containing UVA filters in combination with various UVB filters were evaluated.*

*** Sunscreen Ingredient Abbreviations**

Abbreviation	INCI Name	Abbreviation	INCI Name
BMDM	Butyl Methoxydibenzoylmethane (avobenzone)	HSAL	Homosalate
4-MBC	4-Methylbenzylidene Camphor*	OCTO	Octocrylene
OD-PABA	Octyl dimethyl PABA	OSAL	Octyl Salicylate
OMC	Octyl methoxycinnamate	OT	Octyl Triazone*
TDSA	Terephthalylidene Dicamphor Sulfonic Acid* (ecamsule)	OXY	Oxybenzone
TiO_2	Titanium Dioxide	ZnO	Zinc Oxide

* Note that these ingredients are listed in the EEC Cosmetics Directive 76/768/EEC Appendix VII and are thus approved for use in European sunscreen products. They have not been included in the OTC Sunscreen Drug Products Final Rule dated May 21, 1999 and are not available for use in US commercial OTC sunscreen products.

A. Combinations of 3% Oxybenzone with UVB filters. Combinations of 3% oxybenzone (UVAII filter) with different UVB filters (OMC, OD-PABA and OCTO), their corresponding vehicles, and controls containing only the UVB filters, were tested using the PPD method. The results are summarized in Table 5 and in Figure 5 below. These UVA/UVB combinations yielded UVA-PFs ranging from 2.5 to 3.0, while the vehicle/controls ranged from 1.2 to 2.4. [Refer to Appendix IV for individual subject data.]

Product Code	UV Filter Composition	Mean UVA-PF Value (SD, n)
U-L	Vehicle	**1.2** (0.2, 10)
V-L	OMC 7.5%	**1.7** (0.3, 10)
W-L	OD-PABA 7%	**1.5** (0.2, 10)
X-L	OCTO 7.5%	**2.4** (0.5, 10)
K-L	OMC 7.5% + OXY 3.0%	**2.5** (0.5, 10)
F	OD-PABA 7% + OXY 3.0%	**2.6** (0.8, 10)
I-L	OCTO 7.5% + OXY 3.0%	**3.0** (1.0, 16)

Table 5 - Effect of 3% Oxybenzone in Combination with Various UVB Filters

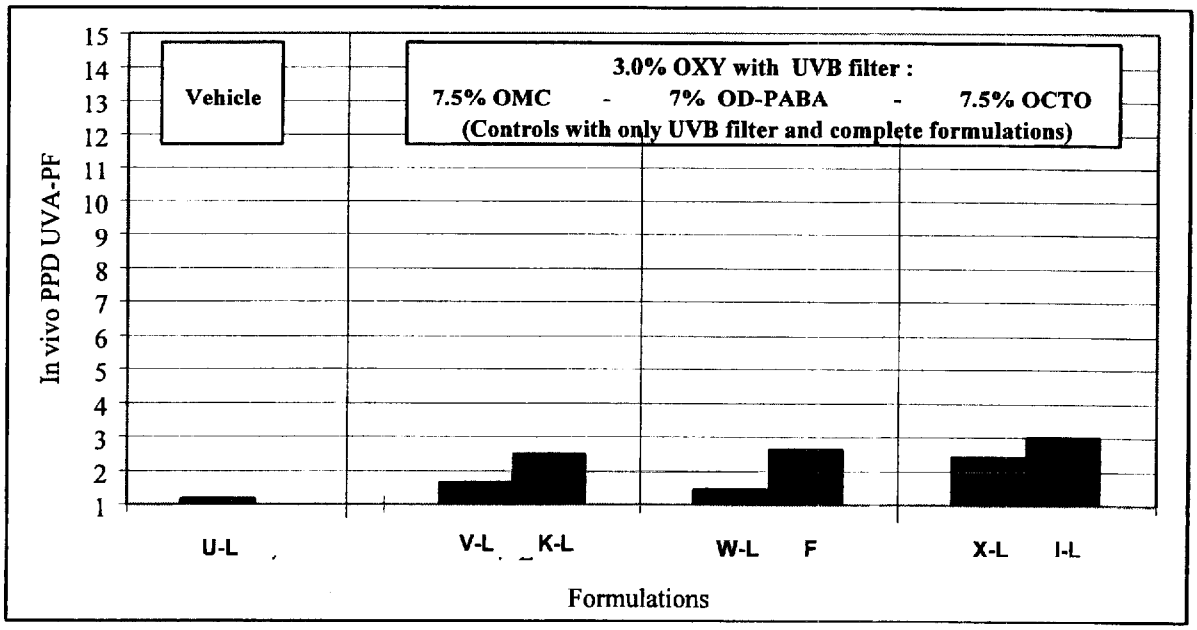

Figure 5 - Effect of 3% Oxybenzone in Combination with Various UVB filters

B. Avobenzone with Octocrylene The results of the evaluation of the UVA-PF of avobenzone in combination with 10% octocrylene are presented in Table 6 and in Figure 6. The UVA protection efficacy of avobenzone is increased when it is combined with

octocrylene[27], as compared to products at the same concentration of avobenzone alone (Figure 3). [Refer to Appendix IV for individual subject data.]

Product Code	Sunscreen Composition	Mean UVA-PF Value (SD, n)
A-L	Vehicle	**1.1** (0.2, 20)
E-L	OCTO 10.0%	**2.0** (0.4, 10)
F-L	OCTO 10.0% + BMDM 1.0%	**4.6** (0.9, 10)
G-L	OCTO 10.0% + BMDM 3.0%	**8.6** (2.5, 10)
H-L	OCTO 10.0% + BMDM 5.0%	**10.6** (2.2, 10)
K-L	OMC 7.5% + OXY 3.0%	**2.5** (0.5, 10)
O-L	OMC 7.5% + OXY 3.0% + BMDM 1.5%	**3.7** (0.7, 10)
P-L	OMC 7.5% + OXY 3.0% + BMDM 3%	**6.0** (1.4, 10)

Table 6 - Dose Effects of BMDM in Combination with Various UVB Filters

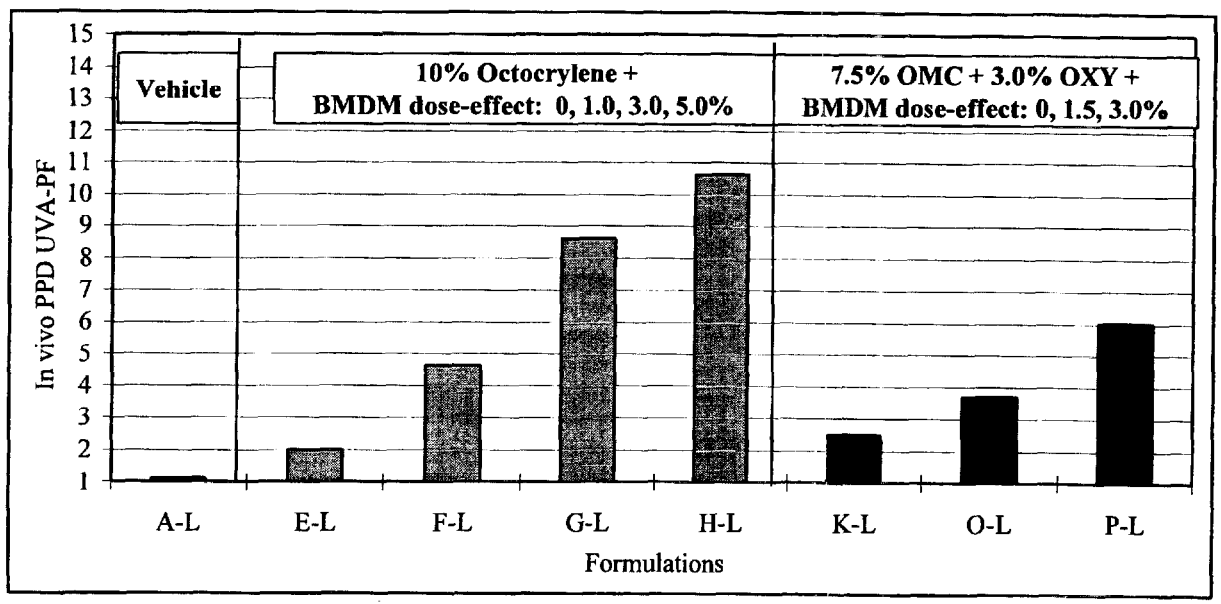

Figure 6 - Dose-effect of BMDM in Combination with Various UVB Filters

C. Avobenzone with OMC and OXY. A dose-response effect is found for avobenzone when used in combination with 7.5% OMC and 3% OXY as shown in Table 6 and Figure 6. However, the UVA protection factors determined in this case (Products O-L and P-L) are lower than those obtained with combinations containing 10% octocrylene as the UVB filter (Products F-L and G-L). [Refer to Appendix IV for individual subject data.]

UVA-PF Assessment of US and European Commercial Products (Labeled SPF15 and SPF30)

Commercial products with identically labeled SPF values were selected from the US and European market in two groups (SPF15 and SPF30) and were evaluated using the PPD method. The results are given in Table 7 and depicted graphically in Figure 7. [Refer to Appendix IV for individual subject data.]

Products	Labeled SPF	UV Filter Combinations	Mean UVA-PF Value (SD, n)
P	15	OMC + ZnO	**1.8** (0.2, 10)
C	15	OMC + OXY	**3.3** (1.2, 10)
J	15	OMC + OXY + BMDM	**4.5** (1.3, 10)
H	15	OMC + OXY + BMDM	**3.7** (0.7, 10)
M	15	OCTO + TDSA + BMDM + TiO_2	**6.8** (1.5, 10)
D	30	OMC + OXY + HSAL	**2.9** (0.8, 10)
O	30	4-MBC + OT + BMDM + TiO_2	**5.3** (1.3, 10)
N	30	OCTO + TDSA + BMDM +TiO_2	**15.9** (3.4, 10)

Table 7: US and European Commercial Products Labeled SPF 15 and 30

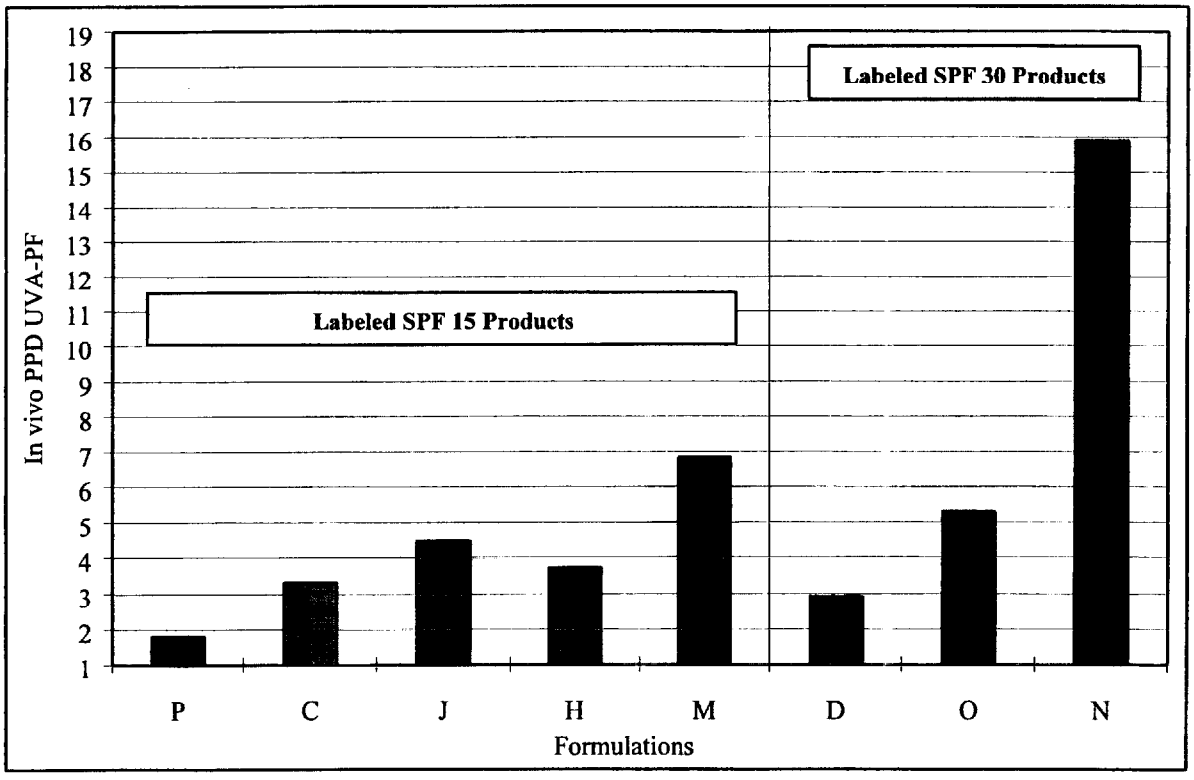

Figure 7: US and European Commercial Products Labeled SPF 15 and 30

DISCUSSION

The principle behind the *in vivo* PPD UVA-PF test method is very similar to that of the *in vivo* SPF test method, in that it relies on a stable biological response (end-point). The UV doses used in PPD testing are equivalent to outdoor sun exposure. Similar to the SPF method, PPD may also be used to assess a product's water resistance.

1. Efficacy spectrum of the UVA source. The UV source used in the PPD method is specific to the UVA portion of the spectrum (Figure 8), i.e. 320-400nm and is identical to that used in the UVA erythema / pigmentation PFA methodology used by Cole[20]. When multiplied by the action spectrum[25] of the PPD skin response, the emission spectrum of the source provides an efficacy spectrum sensitive over the entire UVA waveband including the short UVA wavelengths (UVAII) as shown in Figure 9.

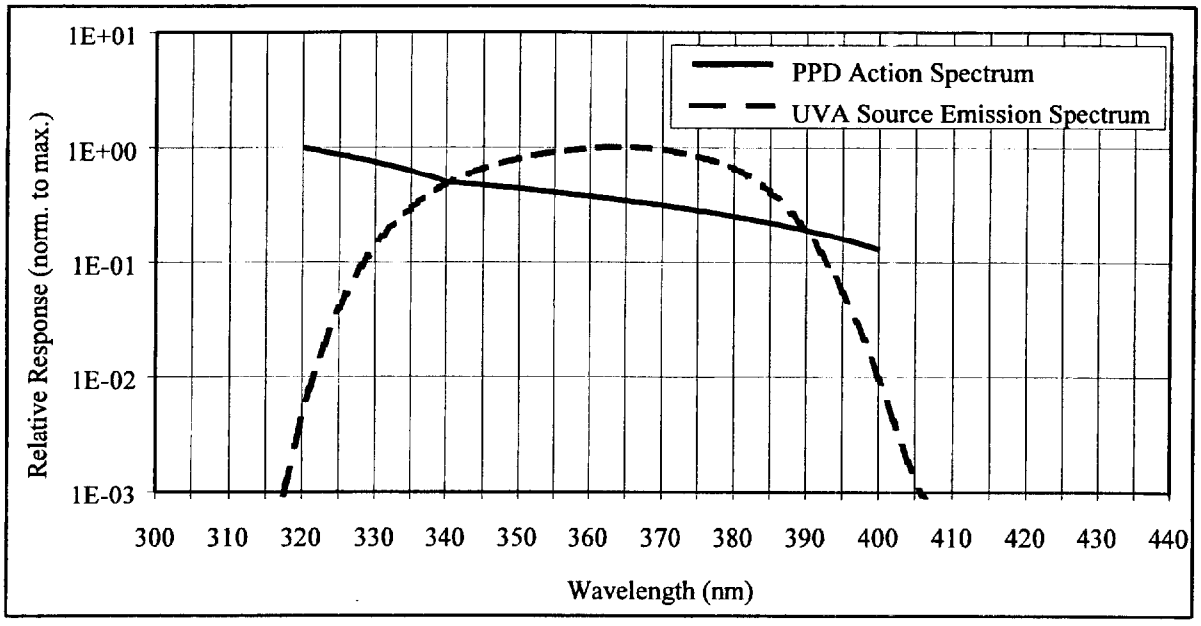

Figure 8: PPD Action Spectrum and Emission Spectrum of the UVA Source

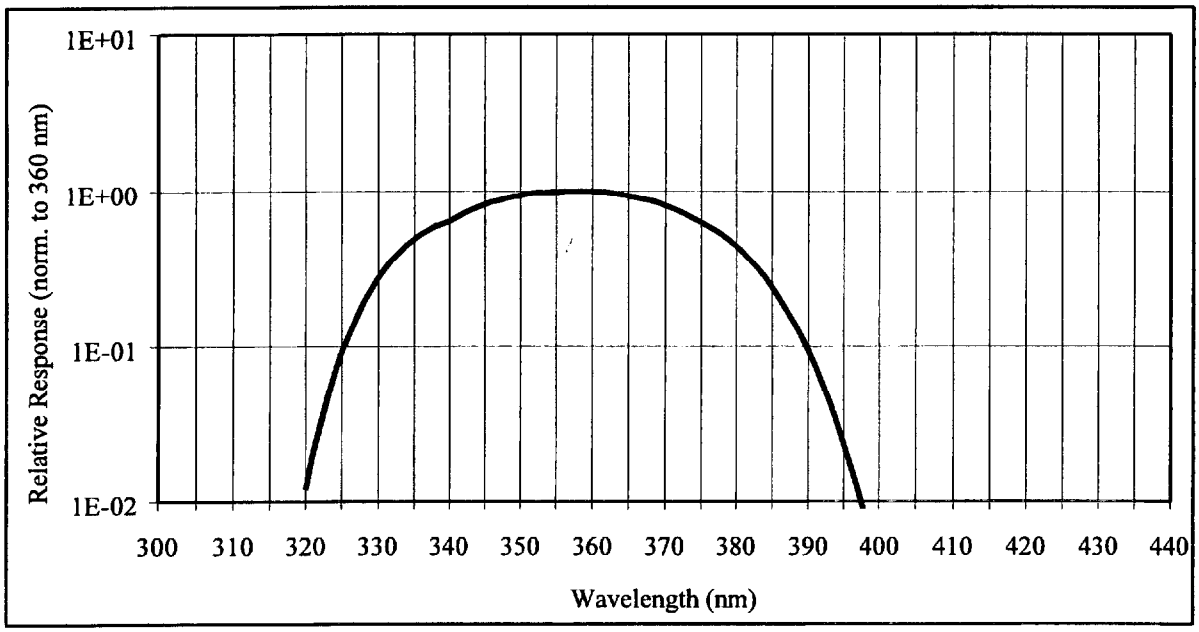

Figure 9: PPD Efficacy Spectrum of the UVA Source

2. Calibration of PPD as an endogenous UVA dosimeter. In Part 1A, it was shown that the PPD method may be used to correctly assess the UVA protection efficacy of the standard neutral density filters. Furthermore, it was shown that this skin response (PPD) is stable, reproducible, sensitive and practically unaffected by the fluence rate. (The fluence rate, or intensity, of the light source was maintained constant, thus the fluence rate after passing through these standard filters was inversely proportional to their attenuation). We are therefore confident that the PPD method of evaluating sun protection of UVA products is a robust and useful approach.

3. Evaluation of the sensitivity of the PPD method relative to the concentration of the active ingredient. We have demonstrated a dose-response effect with concentration of various ingredients: oxybenzone, an effective filter in the UVAII waveband; avobenzone, an effective filter in the UVAI waveband; and ecamsule, a broad UVA filter with maximal absorbance at 345 nm. The results for oxybenzone are very similar to those reported by C. Cole for concentrations of 2% and 5% determined using the UVA erythema/pigmentation PFA method[20]. A dose-response effect from an increase in the physical filter zinc oxide, was demonstrated by the low UVA-PF values observed The data presented herein demonstrate that the PPD method is capable of identifying differences in the concentration levels of different organic and physical filters, irrespective of their wavelength of maximal absorbance in the UVA range.

The question of sensitivity was also addressed by evaluating UVB products that have a minimal absorbance in the UVA range, and by evaluating products containing a mixture of ingredients. The PPD method was used to measure the UVA-PF of filtration systems with increasing complexity: a single UVA filter, oxybenzone, was added to formulations consisting of one of three different UVB filters (Table 5 and Figure 5). The PPD method was found to be sufficiently sensitive in detecting the addition of this filter, which contributes only a small amount of absorbance in the UVAII waveband. A concentration

dependent change in the UVA-PF was also determined using the PPD method when a single UVAI filter (BMDM) was added to a UVB filter (Table 6 and Figure 6). The UVA-PF was also found to increase when the same UVAI filter (BMDM), was added in increasing concentrations to a constant concentration of a UVAII and UVB filter. In all of these combinations of filters (UVAI, UVAII and UVB), the PPD method proved sensitive in detecting changes in UVA-PF.

The PPD method was also shown to be sensitive for products containing a physical filter, zinc oxide, which has a low level of UVA protection efficacy (Products Y-L, Z-L and AZ-L). In comparing UVA-PF values reported in the literature with those reported here, we found that for the CTFA standard sunscreen SPF 15 product (F), a value of 2.6 was determined which is in agreement with the value reported by J. Stanfield[26] using the PPD method. Additionally, for the OMC 7.5% + OXY 3.0% product (K-L) our value of 2.5 is also in agreement with the value reported by N.Lowe[28] using the PPD method.

Assessment of various commercial products demonstrated that the PPD method was sufficiently sensitive to detect differences in UVA-PF values corresponding to different combinations of UVA and UVB filters, even for a product having a very high level of UVA protection as shown in Table 7 and Figure 7.

We have supplied data in Part 1A demonstrating that the PPD phenomenon is linearly dependent on UVA exposure (Table 1). This fact makes the PPD response at 3 ± 1 hour after exposure as reliable, robust and calibratable a skin response as the UVB induced erythema at 24 hours after exposure. Furthermore, the UVA doses necessary to obtain the threshold PPD represent a reasonable sun exposure time of approximately one hour of midday sun.

While the PPD response of the skin does not directly assess a specific UVA risk, it does provide an *in vivo* estimate of the amount of UVA radiation that enters the viable epidermis. The PPD response is equally sensitive throughout the UVA range, as the action spectrum demonstrates. As such, it may be used as an endogenous dosimeter to assess the protection efficacy of products in the UVA range.

Furthermore, due to the persistence of the pigmentation endpoint and to the delayed MPD determination, it is possible to evaluate UVA protection efficacy of colored or pigmented cosmetic products such as lipsticks, make-up and foundations claiming UVA protection, because these products can be completely removed by rinsing during the time lag between exposure and MPD reading, without risk of disturbing the skin color.

In order to evaluate the robustness of the PPD method, we conducted a number of measurements using a group of commercial testing laboratories. The results of these evaluations are presented in Part 2 and clearly support the claim that PPD is a method that is easy to use and leads to reproducible results that are independent of the laboratory in which they were measured.

PART 2: EVALUATION OF THE PPD METHOD AT THREE US COMMERCIAL LABORATORIES

As shown in Part 1, the PPD method based on a stable and visible biological endpoint, i.e., persistent pigment darkening of the skin, is predictive of a product's performance throughout the UVA spectrum. This method addresses the issue of a product's photostability and most importantly, does not overestimate the product's UVA protection.

The PPD method entails the use of a solar simulator equipped with a sufficiently high-energy output to ensure a reasonable exposure time for the subject, as when high SPF values are tested. In light of this potential technical difficulty in the clinical setting, the present multi-center investigation was undertaken to demonstrate the reproducibility of the PPD method under actual use conditions in a commercial laboratory setting.

STUDY OBJECTIVES

The objectives of this study were:

1. To determine the *in vivo* UVA-PF of twelve products containing different OTC category I sunscreen combinations utilizing the PPD method at three independent commercial testing laboratories;

2. To validate the test data by comparing PPD UVA-PF values of test products to those of the sunscreen standard control product at each laboratory;

3. To evaluate the adequacy of the PPD method as a reproducible method for evaluation of a sunscreen's UVA protection efficacy in a commercial test setting.

MATERIALS AND METHODS

Study Design

A multi-center, randomized study was conducted using the JCIA procedure (Appendix I) at each of three commercial laboratories. The sunscreen standard control, formulated as described in the JCIA method, was specific to each laboratory. The sponsor chose not to provide the control sunscreen in order to replicate actual testing conditions. The JCIA procedure is routinely utilized at each investigative site for the evaluation of UVA protection efficacy of commercial sunscreen products. The test products and standard sunscreen control products were evaluated on subjects who served as their own control. The three laboratories followed standard data collection protocols.

Study Sites

1. TKL Research, Inc. (TKL)
   4 Forest Avenue
   Paramus, New Jersey

2. Harrison Research Laboratories (HRL)
   2497 Vauxhall Road
   Union, New Jersey

3. Consumer Product Testing Co. (CPTC)
   70 New Dutch Lane
   Fairfield, New Jersey.

Subject Recruitment and Withdrawal

Subjects were identified using standard telephone screening techniques and screened at the individual study sites. Subjects eligible for inclusion were males or females between the ages of 18 and 65 years in good general health, who were skin types III (burns moderately, tans gradually) or IV (burns minimally, always tans well). Skin in the expected treatment area on the subject's back, i.e., between the waistline and shoulder blade, was to be intact, unblemished and uniform in color. To participate, eligible individuals had to be willing and able to understand and follow all study procedures and restrictions, and to have read, understood, and signed an Informed Consent Form.

Subjects were excluded from study participation, if they:

- Were known to be pregnant or lactating;

- Had a history or presence of any skin disease, medical illness (e.g., diabetes), or skin abnormality (including active dermal lesions, uneven skin tones or scars) that in the opinion of the investigator would interfere with the interpretation of the results or evaluations, affect the safety of the subject, or increase the risk of adverse reaction The presence of small nevi blemishes or moles affecting only a small portion of the test site was acceptable if, in the investigator's opinion, these skin abnormalities would not interfere with the interpretation of results;

- Had a current sunburn or suntan that would interfere with the interpretation of the results;

- Had a history of any systemic diseases with dermal manifestations that may be affected by ultraviolet light exposure (e.g., lupus erythematous);

- Had a history of photosensitivity or photosensitizing illness;

- Had a known hypersensitivity to any known compound in the test product;

- Had been treated within one week prior to the start of the study with any medication (particularly photosensitizing drugs) which may change the body's responses to ultraviolet light and/or interfere with the interpretation of the results and/or affect the safety of the subject. The medications included but were not limited to: thiazides, phenothiazides, antihistamines, antibiotics, corticosteroids, and non-steroidal anti-inflammatory agents or concurrent medications which would interfere with the interpretation of the results or affect the safety of the subject;

- Had a history of toxic or allergic responses to sun exposure.

Subject numbers were assigned utilizing the standard operating procedures of the individual study sites. This number was used to identify the subject on the case report form.

A subject could be withdrawn or be discontinued from the study at any time for any reason. Should this have occurred, the reason for withdrawal was documented on the case report form. The reasons for withdrawal included, but were not limited to, the following:

- The subject requested to be discontinued;

- The investigator felt that continuation in the study was not in the best medical interest of the subject;

- Severe or unexpected adverse reaction occurred;

- The subject did not meet inclusion criteria or was found to have one or more of the exclusion criteria;

- The investigator discontinued the subject for any administrative study related reasons;

- The subject was non-compliant as determined by the investigator.

In addition, the sponsor could terminate the study at any time for any reason.

Test Products

A total of twelve products (Products A through L: 10 commercial suncare products and 2 laboratory formulations) were assessed by the PPD method. [The list of commercial products tested is located in Part 1B under *Test Products*.]

Procedures and Dosing Regimens

Subjects participating in this study were required to make three visits to the test centers, initially for a screening visit followed by a visit to determine the subject's minimal pigment dose (MPD) of unprotected skin, and lastly for the product(s) evaluation visit.

During the screening visit, which occurred within two weeks prior to the first irradiation of unprotected skin, a medical history, including pertinent previous and concurrent medical and skin conditions, medications and allergies, was obtained for each study participant. Further, a skin examination was made of the test site as well as of the arms, face and torso and legs in order to assess the presence of generalized skin or systemic disease. Excess hair on the test site was shaved or clipped with a barber's clipper .

Each investigator received a total of twelve one-ounce jars, labeled in a blinded manner as to the identity of the specific product, but indicating an estimated *in vivo* UVA-PF value and an actual or estimated SPF value. Each investigative site also utilized a sunscreen standard control routinely used by that site. As such, the sunscreen control was allowed to differ from site to site. A member of the investigator's staff who would not be involved in the clinical assessments applied the test product(s) to each subject. Likewise, the staff member who administered the dose of UV radiation was not involved in the clinical assessments.

The MPD of unprotected skin was determined prior to the application of the test products and sunscreen control, and again on the test product(s) evaluation date. Horizontal or vertical rectangular test sites of approximately 50 cm^2 were chosen and outlined with a gentian violet surgical marker, or equivalent, while the subject was in an upright position. This is the position in which irradiation took place. One test site was designated for the test product and sunscreen standard control and an adjacent site was designated for a concurrent MPD determination on untreated skin.

According to the study site's specific randomization, 2.0 mg/cm^2 of the test product(s) and sunscreen standard control were applied to the test sites and were spread evenly over the study areas using a finger cot. After applying the study products and sunscreen control, a waiting period of at least 15 minutes was employed prior to irradiation of the test site(s). During this period, the subject was instructed not to touch his/her back against any surface. One test site remained untreated and served as the area for determining the subject's MPD of unprotected skin. Thus, each study site served as an area for determining the subject's MPD after application of either the test products, the sunscreen standard control, or for determining the subject's MPD when the skin was unprotected.

The subject was irradiated by series of UVA light exposures (expressed as units of time) administered to the sub-sites with the solar simulator. One series of exposures was administered to the untreated, unprotected skin to determine the subject's MPD . The MPD was calculated as the time of exposure that produces minimal pigment darkening at 3 ± 1 hours post exposure.

The protected test sites [sunscreen standard control and test product(s)] were also exposed to UVA. The time intervals selected were calculated from a geometric series represented by $(1.25)^n$ wherein each exposure time interval was twenty five percent greater than the previous time. The exact series of exposures given was determined from the MPD of the unprotected skin and the expected UVA-PF of the test product(s) as provided to each study site. The nominal value of the third sub-site was calculated from

the expected or estimated UVA-PF value of a given test product multiplied by the subject's MPD. The reason for using the geometric sequence of UVA exposure was to maintain the same relative uncertainty (expressed as a constant percentage), independent of the subject's sensitivity to UVA light.

Following the final irradiation and during the time period prior to the observation, the subject remained at rest, avoiding pressure or friction on or against the test sites. The test products were gently wiped off the test sites if required, using a diluted ethanol solution or a cleansing lotion.

UVA Light Source/ Study Site Irradiation

An expert in solar simulation devices calibrated all equipment prior to study initiation (refer to Appendix III).

The three participating laboratories used a 150 watt xenon arc solar simulator, manufactured by Solar Light Co., Philadelphia, PA, as the source of ultraviolet radiation. Two laboratories utilized a single port solar simulator and the third laboratory utilized a multi-port solar simulator. A continuous emission spectrum in the UVA range (320-400 nm) was achieved using a dichroic mirror and Schott WG320 / 1 mm, WG 335 / 3 mm and UG11 / 1 mm filters.

The output of each solar simulator was measured immediately prior to the start of irradiation with an accurately calibrated spectroradiometer system or equivalent instrument. Additionally, the solar simulator output was monitored daily just prior to the first use of the day. Each UV source had no significant time-related fluctuations in radiation emission after an appropriate warm-up time and good beam uniformity, i.e., within 15%.

Monitoring equipment was used to insure appropriate radiation at skin surface during the testing procedure. The flux delivered by the solar simulator at skin level could not exceed 150 mW/cm^2, as measured with a calibrated thermopile, to avoid excess thermal effects. For calculation of the UVA doses, the UVA flux at skin surface was checked using a calibrated UVA meter with cell sensitivity ranging from 320 to 400 nm.

Clinical Measurements/Calculation of UVA-PF

The MPD value of the unprotected and the MPD value of protected skin were determined from a visual observation of the irradiated test sub-sites. All sub-sites in the test area were scored at 3 ± 1 hours post exposure, according to the following Skin Reaction Scoring Scale:

0.0	=	no reaction, no discernible pigment gray/black/brown darkening
0.5	=	barely perceptible (minimal) pigment darkening
1.0	=	unequivocal (moderate) pigment darkening; distinct borders
2.0	=	pronounced or well defined pigment darkening

The lowest dose sub-site within each treatment area showing an unequivocal pigment darkening with distinct borders (scored as 1.0), presented 3 ± 1 hours following the final irradiation, was selected as the MPD value. Different investigators at each test site subjectively determined the sub-site, which attained a Skin Reaction Score of 1.0. The UVA-PF values for the sunscreen standard control and test product(s) were calculated from the exposure time interval producing the MPD of the protected skin and unprotected skin as follows:

$$\text{UVA-PF} = \frac{\text{MPDp}}{\text{MPDu}}$$

Statistical Considerations

The purpose of this study was to evaluate the PPD method for determining the static UVA-PF values of commercial and laboratory sunscreen formulations. The findings with this method were compared to the predicted or estimated UVA-PF based on product composition, labeled active ingredients or sunscreen formulation experience, as well as *in vitro* UVA-PF determinations on similar formulations. Each of the twelve test products (Products A – L) was evaluated utilizing a minimum of 10 subjects at three different commercial laboratories. These three sites were used to assess the reproducibility of the findings across laboratories.

Subjects were unable to be evaluated if either of the following occurred:

- the exposure series failed to elicit a PPD response on unprotected skin or on protected skin sites;
- PPD responses on the protected skin were randomly absent, indicating a lack of uniform treatment application.

The arithmetic mean of the individual UVA-PF values and the associated standard deviation were calculated for each test product and compared to the expected range of UVA-PF based on estimated UVA-PF values determined as previously noted Qualitative comparisons of the mean and standard deviation were made across laboratories. The mean and standard deviation are commonly used measurements to describe the central tendency and dispersion of data in biological and clinical studies. Given the small number of study subjects at each site, the median, 75th percentile, and 25th percentile values were examined as well, since these values can also describe the distribution of the sample data. These values were also compared to the expected ranges of UVA-PF values, and across laboratories to evaluate the sensitivity of the conclusions to different methods of compiling the data. In order to make relative comparisons across the three laboratories, coefficients of variation (CV) were calculated as a method of standardizing the results. A coefficient of variation divides a measure of variability by a measure of central tendency in order to make it more constant over the clinically important range of values. Accordingly, the CV values were calculated using the means and standard deviations as well as using the median and interquartile range (75 th percentile – 25th percentile).

RESULTS

Individual study reports for the three laboratories participating in this study are presented in Appendix V.

This section presents the results of testing in three independent laboratories and summarizes observed results of UVA-PF values generated by the PPD method compared to estimates based on predicted UVA-PF values. Issues of inter- and intra-laboratory variability and reproducibility of test results are also addressed below. The results for each study subject and test product are tabulated and presented in Appendix VI. [Tables 1 – 6 are presented following the conclusion of Part 2]. Data from each laboratory are presented in summary Tables 1 - 3. Table 4 compares the means and standard deviations across the three laboratories. Table 5 addresses the repeatability of the control sunscreen measurements. Table 6 compares results across the three laboratories after standardization of the measurements. Finally, findings based on the medians and 25th and 75th percentiles are discussed in the sections below.

Comparison of Observed to Expected UVA-PF Values

The mean, the range of results within plus or minus one standard deviation of the mean, the range of all observed values, and the number of test subjects are provided in Tables 1-3. One standard deviation was selected as a reasonable indicator of sample variance to account for the potential variability in individual responses to the tested materials since in a normally distributed sample, approximately 68% of the measurements lie within one standard deviation of the mean.

TKL Research, Inc. Test Results

Twelve products were evaluated according to the JCIA procedure to determine their static UVA-PF value. A total of fifty-five subjects between the ages of 19 and 74 completed the study. Three subjects were over the age requirement, but were included with special permission from the Sponsor. Ten subjects per product completed the evaluation of the study products with the exception of product I, for which 11 subjects were tested Among these subjects, a total of 121 measurements were collected based on all the tests with the various products. Out of these 121 measurements, 29 were from individuals with Skin Type IV; the remainder was from individuals with Skin Type III. Table 1 summarizes these results for each of the 12 study products.

As is shown in Table 1, the one-standard deviation range around the observed mean for each product overlapped with each of the ranges of expected UVA-PF values. For eight of the test products, the mean also fell within the estimated UVA-PF range. For four test products (A, C, J and L), the mean fell either slightly above or below the estimated UVA-PF range, but by no more than a factor of 1.15. Similarly, the 25th – 75th percentile range overlapped with the expected ranges of UVA-PF for all of the test products, and the median fell within the expected UVA-PF range for all products except for A, C, and J. Overall, these results indicate a very close correspondence between the measured scores

and the estimated UVA-PF values, particularly considering that individual responses to UV exposure, as well as sunscreen protection products, can vary substantially.

Harrison Research Laboratories, Inc. Test Results

Twelve products were tested according to the JCIA procedure to determine their static UVA-PF value. A total of 10 subjects were recruited ranging in age from 21 to 65. Each product was tested using this same set of individuals. Only subjects with Skin Type III were selected for evaluation by this laboratory. Table 2 summarizes these results.

Table 2 indicates that the one-standard deviation range around the observed mean for each product overlapped with each of the ranges of expected UVA-PF values. For ten of the tested products, the mean also fell within the estimated UVA-PF range. For two products (A and K), the mean fell slightly above the estimated UVA-PF range, but by no more than a factor of 1.1. The 25^{th} – 75^{th} percentile range also overlapped with each expected UVA-PF range, and the median fell within the estimated range for all products except A and K. Again, these results indicate a very close correspondence between the measured scores and the estimated UVA-PF values.

Consumer Product Testing Co. Test Results

Twelve products were tested according to the JCIA procedure to determine their static UVA-PF values. Products A through J were tested with one set of study subjects. One hundred measurements were made on 88 subjects, 16 of which were skin type IV, and the remainder were of skin type III. Products K and L were tested with another set of study subjects. Twenty measurements were made on 20 different subjects, 19 of which were skin type III and 1 was skin type IV. All of the test subjects were between the ages of 18 and 65. A total of 10 subjects were tested for each product except for product A, for which 12 subjects were tested. Table 3 summarizes these results.

As is shown in Table 3, the one-standard deviation range around the observed mean for each product overlapped with each of the ranges of expected UVA-PF values. In addition, the mean result fell within the expected range for each product tested, except for product L which fell slightly below the expected range by a factor of only 1.02. As demonstrated in Tables 1 and 2 for the other two laboratories, these data similarly show a very close correspondence between the measured and expected UVA-PF values. When examining the median, 25^{th} and 75^{th} percentile values for these data, the same patterns of correspondence were found.

These results show an overall pattern of agreement between observed and expected UVA-PF ranges for each product. In addition to demonstrating a correspondence with means and one-standard deviation ranges for each product, the individual observations for each study subject were compared to the expected UVA-PF range. With measurements from all three laboratories taken together, two-thirds of the observed UVA-PF values fell within their corresponding expected UVA-PF range, predicted based on formulation composition and *in vitro* data, and nearly all (96.7%) of the observed measures fell within the expected range, plus or minus 1 UVA-PF.

Inter-laboratory Variability

The Sponsor provided each participating laboratory with a written study protocol, to ensure standard testing procedures in conformance with JCIA procedure. A certain amount of inter-laboratory variability was, however, expected due to study site latitude allowed by the sponsor. Each lab utilized their own equipment and their own research staff. Despite the fact that all equipment was calibrated by an independent expert in light measurement (Appendix III), different equipment utilized by different personnel, and subjective assessment of the Skin Reaction Score by a different investigator at each laboratory, increases the probability of variations despite adherence to the study protocol Furthermore, different types of solar simulators (i.e., single port and multi-port solar simulators) were used at each laboratory. The sunscreen standard controls (each of which differed in UVA-PF strength),and all but one were within the acceptance range specified in the JCIA procedure.* Finally, different sets of test subjects with different breakdowns of skin types (i.e., types III or IV) were evaluated at each lab. This diversity of approaches and test subjects was utilized to replicate real world experience with commercial products, as well as the replication of experiences when individuals are exposed to environmental solar radiation. Accordingly, these factors could result in potentially large differences in the UVA-PF test results.

A compilation of the UVA-PF data across the three laboratories is provided in Table 4. Given the potentially wide variation in individual responses and test measurements that might be expected, these results were, in fact, remarkably consistent. With the exception of Product A, the mean, plus or minus one standard deviation, for each product from each laboratory overlapped one another. With regard to Product A, the magnitude of the difference in the mean, plus or minus one standard deviation, does not appear to be substantial.

The 25th and 75th percentile range was similarly compared across laboratories. For four of the products (A, C, J, and L), all three laboratories did not quite have overlapping ranges. This is likely due to the narrower range provided by the 25th and 75th percentiles since this range encompasses the central 50% of the data whereas the plus or minus one standard deviation from the mean range encompasses the central 68% of the data.

Reproducibility of the UVA-PF Measure

The reproducibility of the PPD method for measuring UVA-PF can be evaluated by examining the variability of results for the control products used by each investigative site as well as the variability of results across individuals for each test product within each site.

A sunscreen standard control product of known UVA-PF, formulated in accordance with the JCIA procedure, was used as a control for each subject and was tested with each of the twelve test products in each laboratory. For TKL Research, the estimated UVA-PF of

* The mean PFA value of the sunscreen standard control used at TKL Research Inc. was slightly higher than the acceptable range for all products tested.

the control product was not stated, however, the mean observed UVA-PF as reported by the laboratory was 5.40. Consumer Product Testing Co. used a control product with an estimated UVA-PF of 3.75 and the Harrison Research Laboratory used a control product with an estimated UVA-PF of 4.00.

Table 5 summarizes all the data reported from repeated measurements of the control product by each of the three laboratories. The mean and standard deviation, and the range associated with the mean, plus or minus one standard deviation, are shown for each of the 12 products, based on all subjects tested at each laboratory. The mean reported results for TKL ranged within only a factor of 1.3 across tested products, from 4.75 to 5.97. The mean results for CPTC also ranged within a factor of 1.2, from 3.59 to 4.24. The mean results for HRL ranged within a factor of 1.1, from 4.1 to 4.6. The ranges of reported results within plus or minus one standard deviation for each product all substantially overlapped one another for each laboratory. These findings were supported through the same assessment of the median and $25^{th} - 75^{th}$ percentile range. In summary, within each laboratory, the measured UVA-PF of each test product was extremely consistent across evaluations, demonstrating the reproducibility of the PPD method for measuring UVA-PF.

The robustness of the test measurement can also be demonstrated by examining the coefficient of variation (CV) within each laboratory across individuals tested for each product. The coefficient of variation expresses sample variability relative to a measure of central tendency and, because it is a relative measure, is divorced from the actual magnitude of the reported test measurements. The coefficient of variation reported in Table 6 was calculated by dividing the standard deviation by the mean, and is reported for each test product examined in each laboratory. As can be seen, the CV values range from 15% to 33% for TKL, 12% to 22% for HRL, and 14% to 29% for CPTC. A second coefficient of variation was also calculated by dividing the interquartile range (75^{th} percentile value minus the 25^{th} percentile value) by the median. This method provided a wider range of CV values, most likely due to the small sample sizes and reliance on the exact sampling distribution. These CV values ranged from 20% to 43% for TKL, 0 to 50% for HRL and 0 to 40% for CPTC. A CV value of 0 indicates that the central 50% of the observed sample were identical, and therefore very reproducible, while the larger CV values may simply reflect the skewness associated with a small sample of data.

As noted above, individual responses to both sun exposure and sunscreen effectiveness, even within groups with similar skin types, are likely to vary substantially. Additional uncertainty and measurement error is introduced by the variation that exists in equipment and personnel at each laboratory. Given this backdrop, the CV results suggest no substantial variation with repeated testing of each product across individuals in each laboratory, and indicate that the PPD method provides a robust approach for measuring UVA-PF.

DISCUSSION

The current focus on erythema protection as the standard for the evaluation of sunscreen products has led many to erroneously conclude that erythema prevention is the only important goal of sunscreen protection. Based on the fact that the untoward effects of UVA exposure are not adequately addressed by the SPF test method that measures a product's erythemal protection, L'Oréal Research conducted this study to demonstrate that the persistent pigment darkening (PPD) method is a reliable and reproducible technique for determination and classification of UVA protection.

Using the PPD method, the individual UVA Protection Factor (UVA-PF) of a sunscreen product is defined as the ratio of the UVA doses necessary to induce the same minimal pigment darkening effect on sunscreen protected skin and on unprotected skin. The clinical endpoint is the persistent pigment darkening of the skin observed at 3 ± 1 hour following UVA exposure in a panel of selected human volunteers. The mean UVA-PF of the product is the arithmetic mean of the individual UVA-PF measurements determined on the panel.

The findings of this evaluation demonstrate that the PPD method consistently results in the generation of a UVA-PF score comparable to the predicted UVA-PF value based on knowledge of the formulation's active ingredients and prior UVA-PF determinations of similar formulations. In all cases, at each of the three testing sites, the results indicate a very close correspondence between the measurements using the PPD method and the estimated UVA-PF. Since the expected UVA-PF value for each of the products is predicted based on the product formulation experience and does not represent analytical results, quantitative analysis of variation between the predicted and observed ranges could not be conducted. However, evaluation of the overlap of one standard deviation around the mean of the observed results (as well as the 25^{th} to 75^{th} percentile range), to the estimated ranges, demonstrates good correspondence.

While an effort was made to ensure that each of the investigative sites performed their evaluation similarly, variation between laboratories was expected and encouraged. While the solar simulators at each of the three sites were calibrated to provide a consistent UVA exposure, there were differences in the equipment, (single port simulator used by two sites, multi-port simulator used by the third site) and the sunscreen controls used. In fact, each laboratory used its own sunscreen standard control as an internal control. Likewise, differences in personnel involved in product application and rating of pigment darkening were expected. Based on these anticipated differences in study conduct, the comparable findings across the laboratories demonstrate the viability of the method.

Table 1 - Observed and Expected UVA-PF Values for Individual Study Products
TKL Research, Inc.

Product ID	Number of Subjects	Labeled SPF	Expected UVA-PF* based on SPF Label	Observed UVA-PF* mean ± 1 standard deviation (Range based on 1 standard deviation)	Range of Observed Values
Product A	10	SPF 4	2 - 3	1.74 ± 0.26 (1.48 - 2.00)	1.25 - 2.00
Product B	10	SPF 8	2 - 3	2.25 ± 0.52 (1.73 - 2.77)	1.60 - 3.13
Product C	10	SPF 15	3 - 5	2.83 ± 0.44 (2.39 - 3.27)	2.40 - 3.75
Product D	10	SPF 30	3 - 6	4.85 ± 0.88 (3.97 - 5.73)	3.99 - 6.26
Product E	10	SPF 45	3 - 6	5.41 ± 1.41 (4.00 - 6.82)	3.20 - 7.83
Product F	10	SPF 15	3 - 4	3.25 ± 0.59 (2.66 - 3.84)	2.56 - 4.00
Product G	10	estimated SPF 15	3 - 6	5.22 ± 1.05 (4.17 - 6.27)	3.20 - 6.25
Product H	10	SPF 15	4 - 7	5.08 ± 0.87 (4.21 - 5.95)	3.84 - 6.00
Product I	11	SPF 15	4 - 6	4.04 ± 1.05 (2.99 - 5.09)	2.55 - 6.25
Product J	10	SPF 15	4 - 7	3.75 ± 0.97 (2.78 - 4.72)	2.32 - 5.69
Product K	10	estimated SPF 7	2 - 3	2.41 ± 0.80 (1.61 - 3.21)	1.28 - 3.91
Product L	10	estimated SPF 7	6 - 7	7.36 ± 1.90 (5.46 - 9.26)	6.00 - 11.73

*** UVA-PF = MPPD Protected Skin / MPPD Unprotected Skin**

Table 2 - Observed and Expected UVA-PF Values for Individual Study Products Harrison Research Laboratories, Inc.

Product ID	Number of Subjects	Labeled SPF	Expected UVA-PF* based on SPF Label	Observed UVA-PF* mean ± 1 standard deviation (Range based on 1 standard deviation)	Range of Observed Values
Product A	10	SPF 4	2 - 3	3.19 ± 0.62 (2.57 - 3.81)	2.40 - 3.75
Product B	10	SPF 8	2 - 3	2.97 ± 0.49 (2.48 - 3.46)	2.40 - 3.75
Product C	10	SPF 15	3 - 5	3.98 ± 0.79 (3.19 - 4.77)	3.20 - 5.00
Product D	10	SPF 30	3 - 6	4.97 ± 0.98 (3.99 - 5.95)	4.00 - 6.25
Product E	10	SPF 45	3 - 6	5.40 ± 0.63 (4.77 - 6.03)	4.80 - 6.00
Product F	10	SPF 15	3 - 4	3.82 ± 0.85 (2.97 - 4.67)	3.20 - 5.00
Product G	10	estimated SPF 15	3 - 6	4.95 ± 0.82 (4.13 - 5.77)	4.00 - 6.25
Product H	10	SPF 15	4 - 7	6.51 ± 1.13 (5.38 - 7.64)	4.80 - 7.50
Product I	10	SPF 15	4 - 6	5.05 ± 0.75 (4.30 - 5.80)	4.00 - 6.25
Product J	10	SPF 15	4 - 7	5.43 ± 0.92 (4.51 - 6.35)	4.80 - 7.50
Product K	10	estimated SPF 7	2 - 3	3.31 ± 0.50 (2.81 - 3.81)	2.40 - 3.75
Product L	10	estimated SPF 7	6 - 7	7.00 ± 1.57 (5.43 - 8.57)	5.60 - 8.75

*** UVA-PF = MPPD Protected Skin / MPPD Unprotected Skin**

Table 3 - Observed and Expected UVA-PF Values for Individual Study Products Consumer Product Testing Co.

Product ID	Number of Subjects	Labeled SPF	Expected UVA-PF* based on SPF Label	Observed UVA-PF* mean ± 1 standard deviation (Range based on 1 standard deviation)	Range of Observed Values
Product A	12	SPF 4	2 - 3	2.62 ± 0.43 (2.19 - 3.05)	1.92 - 3.00
Product B	10	SPF 8	2 - 3	2.55 ± 0.49 (2.06 - 3.04)	1.92 - 3.75
Product C	10	SPF 15	3 - 5	3.34 ± 0.68 (2.26 - 4.02)	2.05 - 4.01
Product D	10	SPF 30	3 - 6	4.22 ± 0.59 (3.63 - 4.81)	3.20 - 5.00
Product E	10	SPF 45	3 - 6	4.17 ± 0.72 (3.45 - 4.89)	3.07 - 4.08
Product F	10	SPF 15	3 - 4	3.64 ± 0.69 (2.95 - 4.33)	2.56 - 5.00
Product G	10	estimated SPF 15	3 - 6	4.44 ± 0.87 (3.57 - 5.31)	3.19 - 6.25
Product H	10	SPF 15	4 - 7	5.20 ± 0.91 (4.29 - 6.11)	3.83 - 6.00
Product I	10	SPF 15	4 - 6	4.09 ± 0.90 (3.19 - 4.99)	2.56 - 5.00
Product J	10	SPF 15	4 - 7	5.43 ± 1.45 (3.98 - 6.88)	3.07 - 7.50
Product K	10	estimated SPF 7	2 - 3	2.91 ± 0.53 (2.38 - 3.44)	2.40 - 3.75
Product L	10	estimated SPF 7	6 - 7	5.78 ± 1.65 (4.13 - 7.43)	4.47 - 8.74

*** UVA-PF = MPPD Protected Skin / MPPD Unprotected Skin**

Table 4 - Comparison of Expected UVA-PF to Observed UVA-PF (mean +/- 1 standard deviation)

Product ID	Labeled SPF	Estimated UVA-PF	Mean Observed UVA-PF Score ± 1 Standard Deviation		
			TKL Research	CPTC	HR Laboratory
Product A	SPF 4	2 - 3	1.74 (1.48 - 2.00)	2.62 (2.19 - 3.05)	3.19 (2.57 - 3.81)
Product B	SPF 8	2 - 3	2.25 (1.73 - 2.77)	2.55 (2.06 - 3.04)	2.97 (2.48 - 3.46)
Product C	SPF 15	3 - 5	2.83 (2.39 - 3.27)	3.34 (2.26 - 4.02)	3.98 (3.19 - 4.77)
Product D	SPF 30	3 - 6	4.85 (3.97 - 5.73)	4.22 (3.63 - 4.81)	4.97 (3.99 - 5.95)
Product E	SPF 45	3 - 6	5.41 (4.00 - 6.82)	4.17 (3.45 - 4.89)	5.40 (4.77 - 6.03)
Product F	SPF 15	3 - 4	3.25 (2.66 - 3.84)	3.64 (2.95 - 4.33)	3.82 (2.97 - 4.67)
Product G	estimated SPF 15	3 - 6	5.22 (4.17 - 6.27)	4.44 (3.57 - 5.31)	4.95 (4.13 - 5.77)
Product H	SPF 15	4 - 7	5.08 (4.21 - 5.95)	5.20 (4.29 - 6.11)	6.51 (5.38 - 7.64)
Product I	SPF 15	4 - 6	4.04 (2.99 - 5.09)	4.09 (3.19 - 4.99)	5.05 (4.30 - 5.80)
Product J	SPF 15	4 - 7	3.75 (2.78 - 4.72)	5.43 (3.98 - 6.88)	5.43 (4.51 - 6.35)
Product K	estimated SPF 7	2 - 3	2.41 (1.61 - 3.21)	2.91 (2.38 - 3.44)	3.31 (2.81 - 3.81)
Product L	estimated SPF 7	6 - 7	7.36 (5.46 - 9.26)	5.78 (4.13 - 7.43)	7.00 (5.43 - 8.57)

Table 5
Repeated Measures of Control Product

Standard Product		TKL	CPTC	HRL
With Product A	Mean	5.53	4.14	4.50
	Standard Deviation	0.82	0.49	0.53
	Range (Mean ± 1 Standard Deviation)	4.71 - 6.35	3.65 - 4.63	3.97 - 5.03
With Product B	Mean	5.97	3.96	4.40
	Standard Deviation	1.24	0.56	0.52
	Range (Mean ± 1 Standard Deviation)	4.73 - 7.21	3.40 - 4.52	3.88 - 4.92
With Product C	Mean	5.56	4.05	4.20
	Standard Deviation	1.08	0.60	0.42
	Range (Mean ± 1 Standard Deviation)	4.48 - 6.64	3.45 - 4.65	3.78 - 4.62
With Product D	Mean	5.53	3.86	4.10
	Standard Deviation	0.82	0.49	0.32
	Range (Mean ± 1 Standard Deviation)	4.71 - 6.35	3.37 - 4.35	3.78 - 4.42
With Product E	Mean	5.59	4.22	4.20
	Standard Deviation	1.20	0.49	0.42
	Range (Mean ± 1 Standard Deviation)	4.39 - 6.79	3.73 - 4.71	3.78 - 4.62
With Product F	Mean	4.88	3.96	4.10
	Standard Deviation	1.03	0.56	0.32
	Range (Mean ± 1 Standard Deviation)	3.85 - 5.91	3.40 - 4.52	3.78 - 4.42
With Product G	Mean	5.46	3.92	4.30
	Standard Deviation	1.18	0.92	0.48
	Range (Mean ± 1 Standard Deviation)	4.28 - 6.64	3.00 - 4.84	3.82 - 4.78
With Product H	Mean	4.83	3.59	4.50
	Standard Deviation	0.93	0.32	0.53
	Range (Mean ± 1 Standard Deviation)	3.90 - 5.76	3.27 - 3.91	3.97 - 5.03
With Product I	Mean	5.44	3.95	4.60
	Standard Deviation	1.23	0.56	0.52
	Range (Mean ± 1 Standard Deviation)	4.21 - 6.67	3.39 - 4.51	4.08 - 5.12
With Product J	Mean	5.46	4.24	4.50
	Standard Deviation	1.18	0.72	0.53
	Range (Mean ± 1 Standard Deviation)	4.28 - 6.64	3.52 - 4.96	3.97 - 5.03
With Product K	Mean	5.20	4.24	4.40
	Standard Deviation	1.00	0.72	0.52
	Range (Mean ± 1 Standard Deviation)	4.20 - 6.20	3.52 - 4.96	3.88 - 4.92
With Product L	Mean	5.64	4.06	4.40
	Standard Deviation	1.47	0.71	0.52
	Range (Mean ± 1 Standard Deviation)	4.17 - 7.11	3.35 - 4.77	3.88 - 4.92

Table 6 - Coefficients of Variation of Repeated Measures of Test Products, By Laboratory

Test Material	TKL Research	HR Laboratory	CPTC
Product A	15%	19%	16%
Product B	23%	17%	19%
Product C	16%	20%	20%
Product D	18%	20%	14%
Product E	26%	12%	17%
Product F	18%	22%	19%
Product G	20%	17%	20%
Product H	17%	17%	18%
Product I	26%	15%	22%
Product J	26%	17%	27%
Product K	33%	15%	18%
Product L	26%	22%	29%

Coefficient of Variation = Standard Deviation / Mean X 100

OVERALL CONCLUSIONS

Persistent Pigment Darkening (PPD) is a stable skin response that is linearly dependent on the amount of UVA radiation that enters the viable epidermis The PPD is practically independent of the fluence rate Therefore, PPD may serve as an *in vivo* endogenous dosimeter to assess the protection efficacy of products in the UVA. As in the case of SPF testing, PPD tests are carried out on humans using realistic UV exposures.

The PPD response, when integrated into a method to assess the protection efficiency of UVA products, is sensitive and specific to all UVA wavelengths The PPD method was shown to be equally sensitive to all UVA filtration schemes.

The PPD method is robust and reproducible and may be easily implemented in laboratories that are familiar with SPF testing.

We therefore propose that the PPD method be considered as an acceptable and recommended method for assessing the UVA efficacy of sun protection filters.

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